

Genotypic/phenotypic variation in target host and microbial agents - implications for microbial control.



Outline

- When does phenotypic or genotypic variation matter?
- Improvements that can be made in understanding and coping with genetic diversity

When does phenotypic or genotypic variation matter?

- In targets
 - Resistance, tolerance
 - Multiple introductions of diverse lineages
 - Evolution
 - Replacement



When does phenotypic or genotypic variation matter?

- Pathogen agent
 - Differences in failures in establishment or efficacy
 - Evolution
 - 29% of pathogens used for classical biological control considered successful (Charudattan. 2005. Biological Control 35, 183-196)

When does phenotypic or genotypic variation matter?

- Tight host-specificity usually more common in pathogen agents than arthropod agents due to intimate evolution with hosts (Morin et al. 2006. Aust. J. Ent.)
- Host-specificity, a two-edged sword



Diversity in Plants

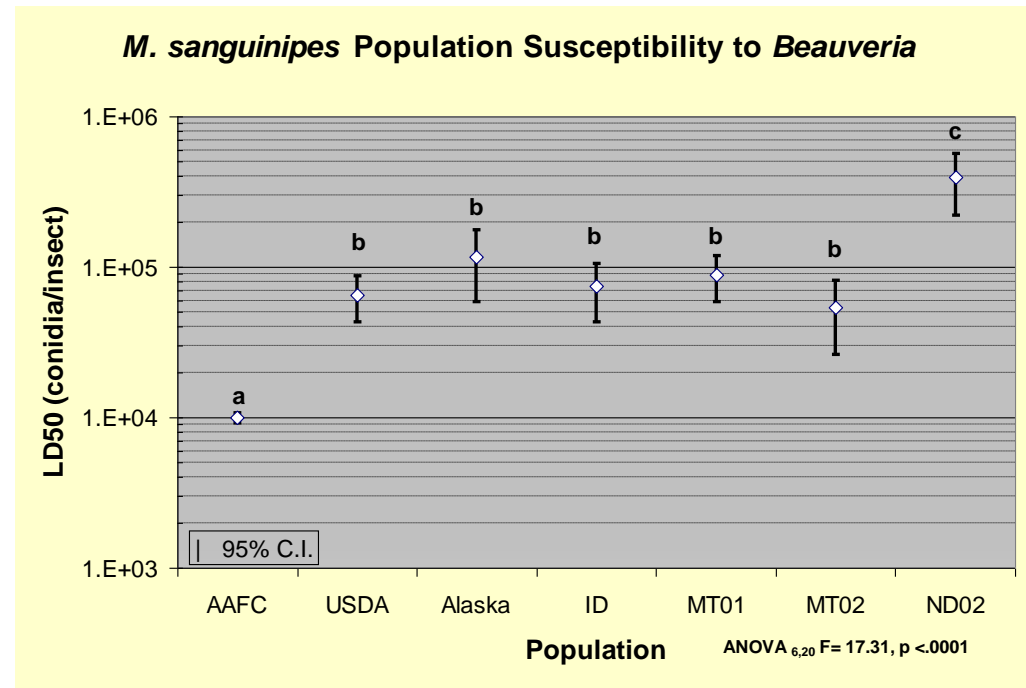
- Heritable resistance or tolerance to disease common
- Rush skeletonweed/ *Puccinia chondrillina*
 - Chaboudez and Burdon. Frequency-dependent selection in a wild plant-pathogen system. 1995. Oecologia



Eric Coombs, www.forestryimages.org

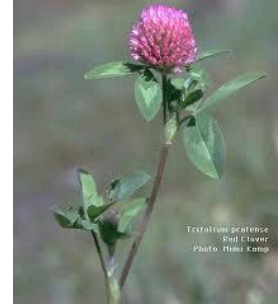
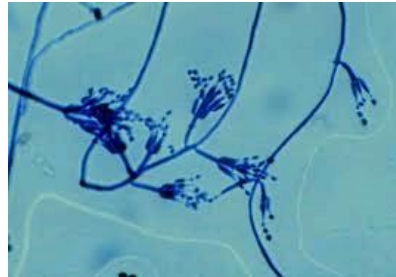
Diversity in Arthropods

- *Melanoplus sanguinipes*
 - Differences in susceptibility to *Beauveria bassiana* strain GHA (Jaronski and Schlothauer, USDA ARS Sidney, MT)



Diversity in Arthropods

- *Acyrtosiphon pisum*
 - Specializes on various plant species
- *Lotus uliginosus* (susceptible) and *Trifolium pratense* (resistant)



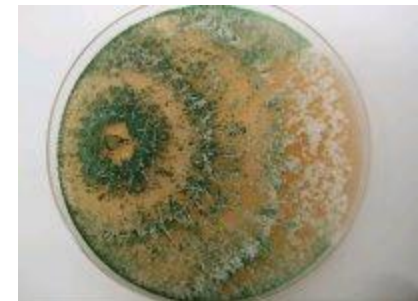
- Fungal pathogen: *Erynia neoaphidis*
- Ferrari and Godfray. 2003. Resistance to a fungal pathogen and host plant specialization in the pea aphid. Ecology Letters .

Diversity in Pathogens

- Destructive mycoparasitism
 - Tondje et al. 2007. Isolation and identification of mycoparasitic isolates of *Trichoderma asperellum* with potential for suppression of black pod disease of cacao in Cameroon. Biological Control.
 - 4/200 isolates capable of necrotrophic mycoparasitism



USDA ARS EBCL



When should we look for genotype diversity?

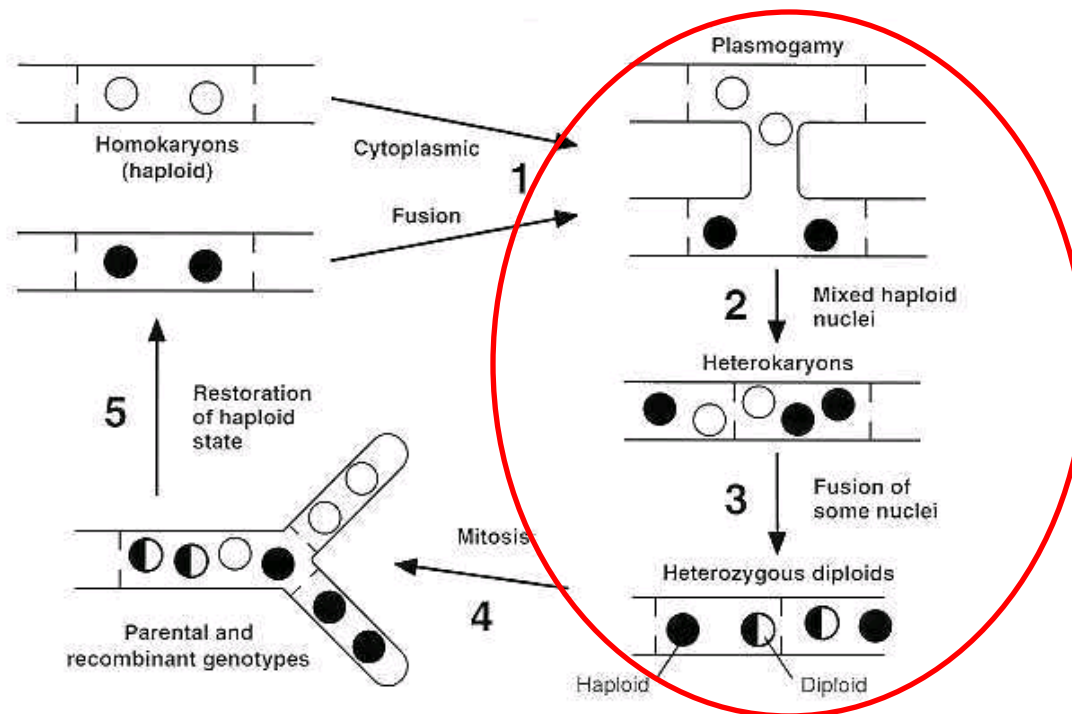
- When phenotypic differences in host-specificity, efficacy, etc. are discovered.
- At the start of a biological control project
 - Subject the potential agent to a representation of the continuum of diversity in the target.
 - Small genetic study of invasion diversity before exploration

Coping with diversity

- Clonal target
 - Fewer target lineages (usually)
 - Easier to determine origins
 - Less chance of evolution of resistance/tolerance
- Sexual target
 - Many target lineages (unless extremely low #s in founding event or strong bottleneck)
 - Gene flow can erase evidence of origins
 - Gene flow leads to higher rates of evolution of resistance/tolerance

Coping with Diversity

- Pathogen Agents
 - Homokaryotic vs. heterokaryotic



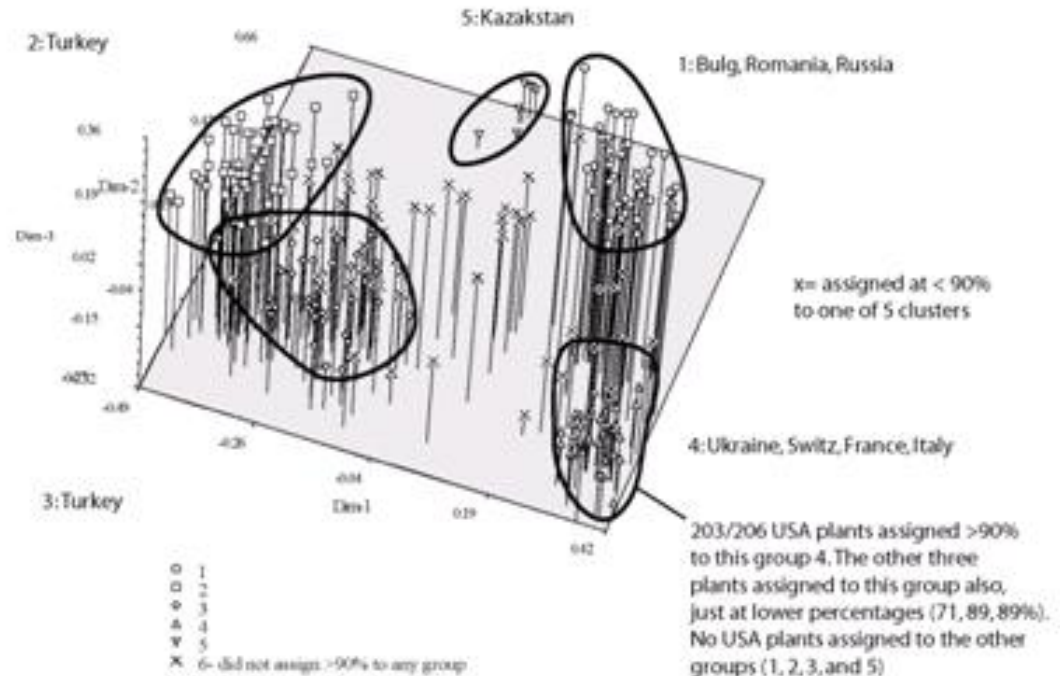
Improvements that can be made in understanding and coping with genetic diversity

- Utilize higher resolution genetic markers
 - SSRs AFLPs ISSRs SNPs Sequences microarrays
 - Some of these are cheap
 - Some can be used as bar-codes
- Faster data collection and analysis
 - Determine population structure of invasions
 - Better sampling of native range
- Genetic diversity often cryptic

Improvements

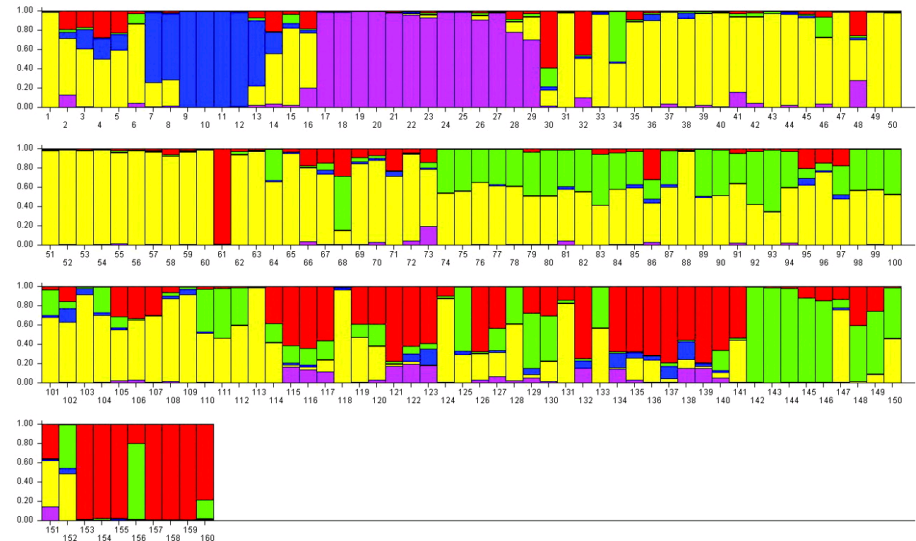
- Bayesian assignment software
 - Determine number of genetic clusters
 - Determine origin of genotypes

457 plants, 124 AFLP loci



Improvements

- Bayesian assignment software
 - Determine number of clusters
 - Determine origin of genotypes
- **Structure.** Falush et al. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Mol Ecol Notes
 - Quantitative assignment
 - Dominant markers ok!
 - AFLP, ISSR



Improvements

- Softlinks Phylogenetic and Population Genetic Software: <http://research.amnh.org/users/koloko/softlinks/>
- The Genetic Software Forum: <http://gsf.gc.ucdavis.edu/>

Genetic diversity and distribution of rush skeletonweed (*Chondrilla juncea* L.; Asteraceae) world-wide

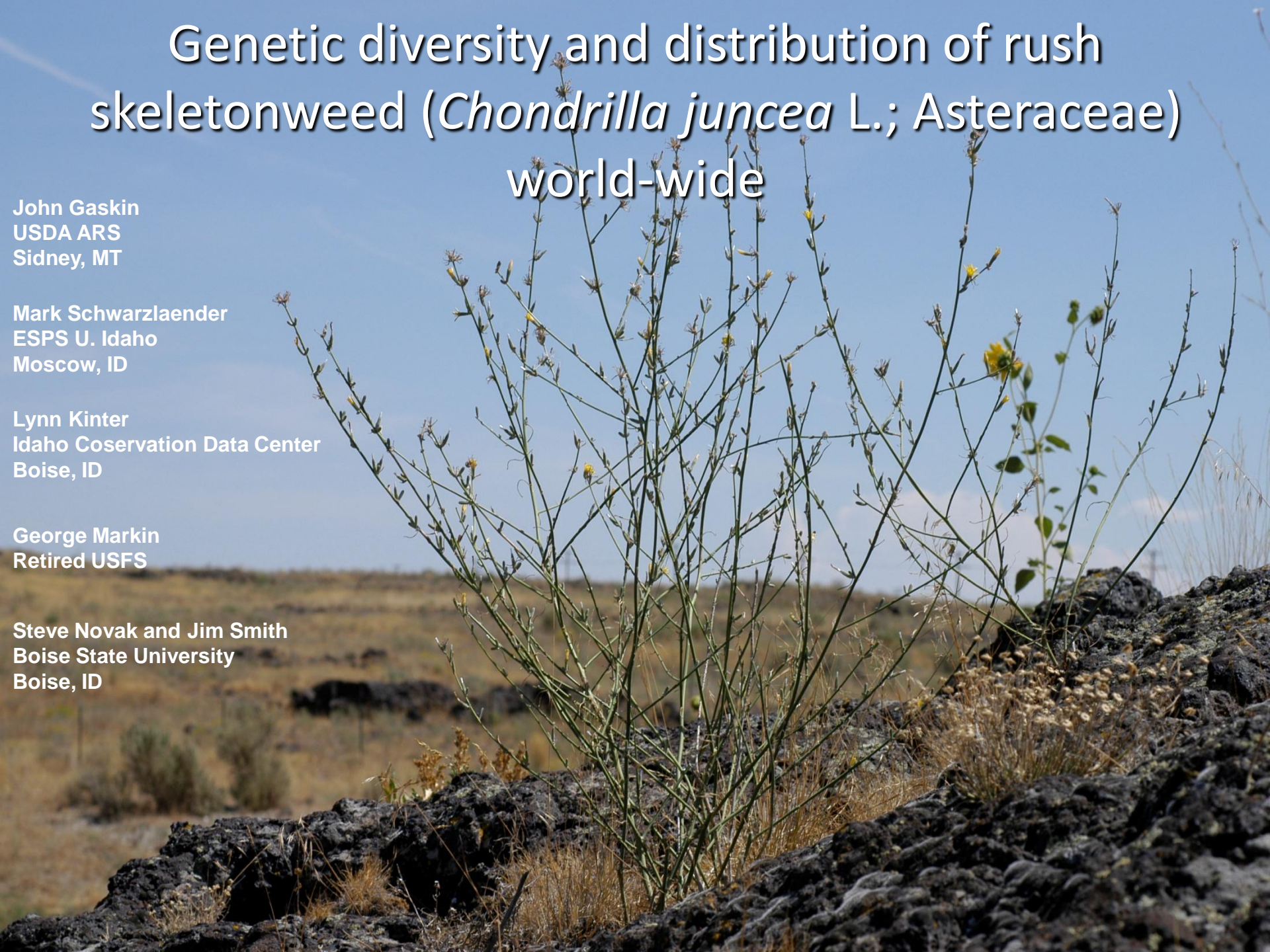
John Gaskin
USDA ARS
Sidney, MT

Mark Schwarzlaender
ESPS U. Idaho
Moscow, ID

Lynn Kinter
Idaho Conservation Data Center
Boise, ID

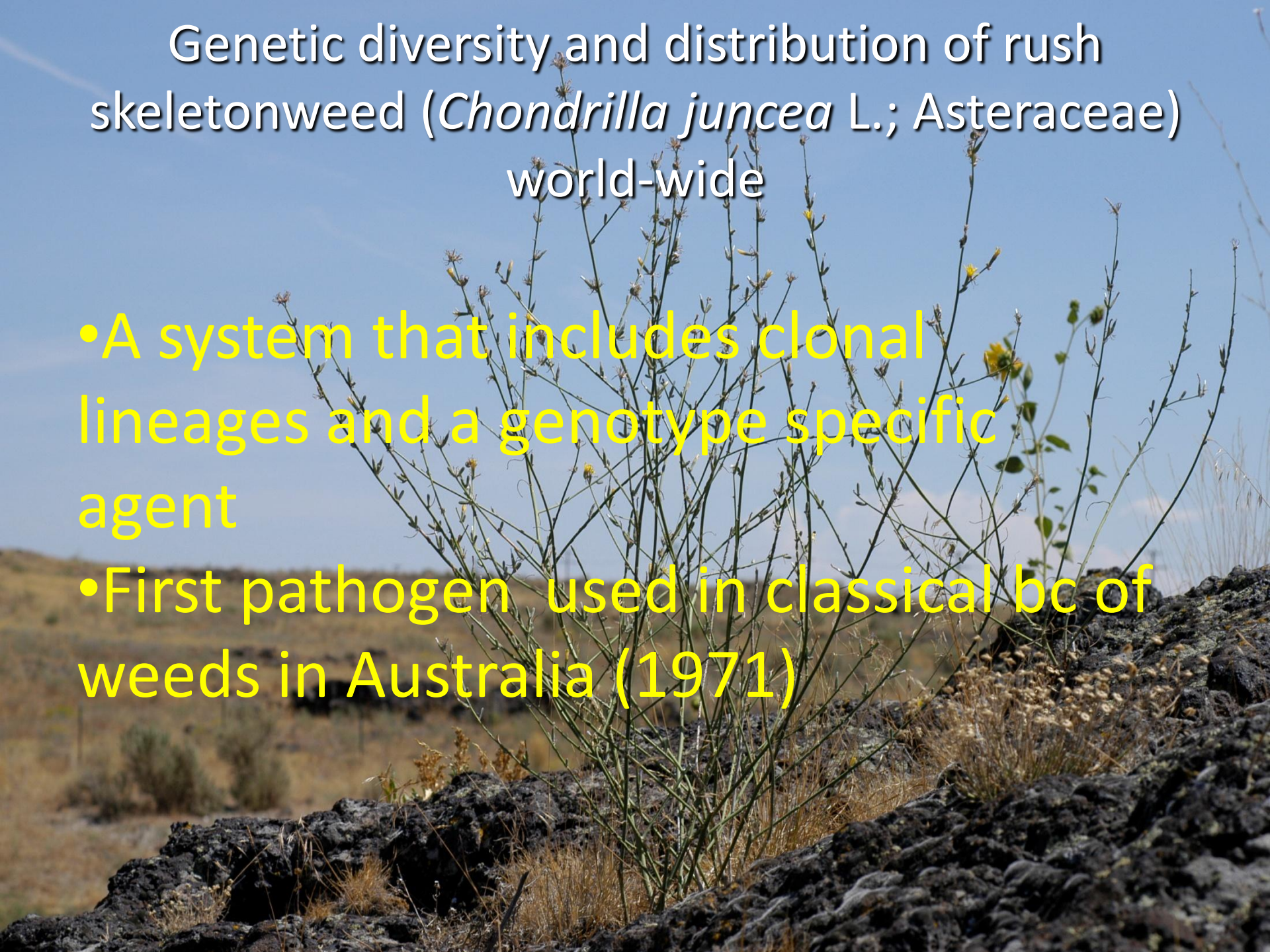
George Markin
Retired USFS

Steve Novak and Jim Smith
Boise State University
Boise, ID



Genetic diversity and distribution of rush skeletonweed (*Chondrilla juncea* L.; Asteraceae) world-wide

- A system that includes clonal lineages and a genotype specific agent
- First pathogen used in classical bc of weeds in Australia (1971)





Life cycle

- Perennial
- Apomictic (obligate) triploids in USA
- Some native pops sexual (diploid)
- Spreads by seed and rhizome

CHJU

\$3.5 million per year for eradication in W Australia



rush skeletonweed rust (*Puccinia chondrillina*)

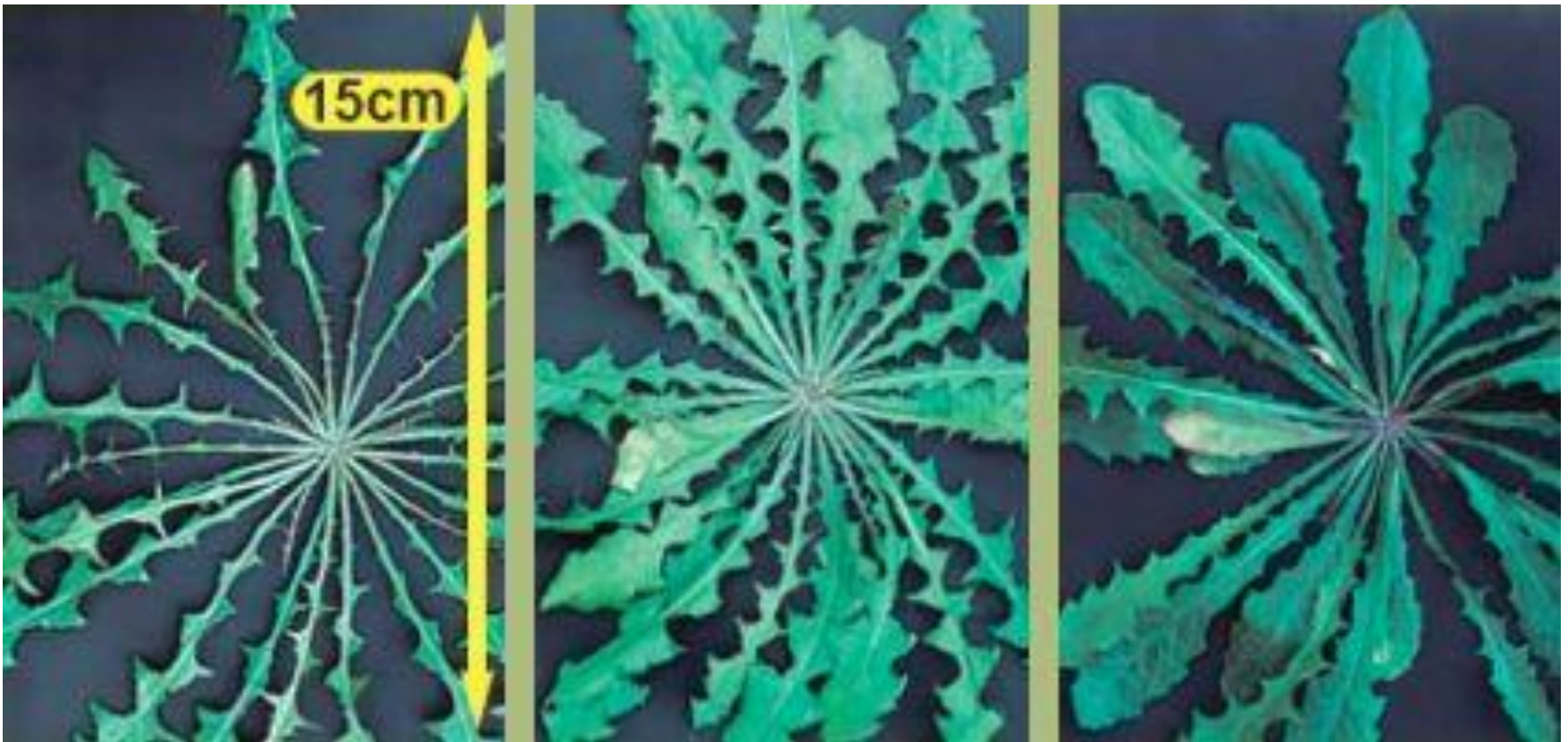


Current status of biocontrol

- Some types of rush skeletonweed resistant to some agents
 - Three forms in Australia, one controlled (?) by the rust
 - Since then, the other forms increased in distribution and abundance
 - One form in USA resistant to rust
 - Rust strains are cryptic, so distribution unknown

Phenotypes in Australia

Rosette width and leaf width, enzymes



Phenotypes in USA

Height, location, phenology, (enzymes)

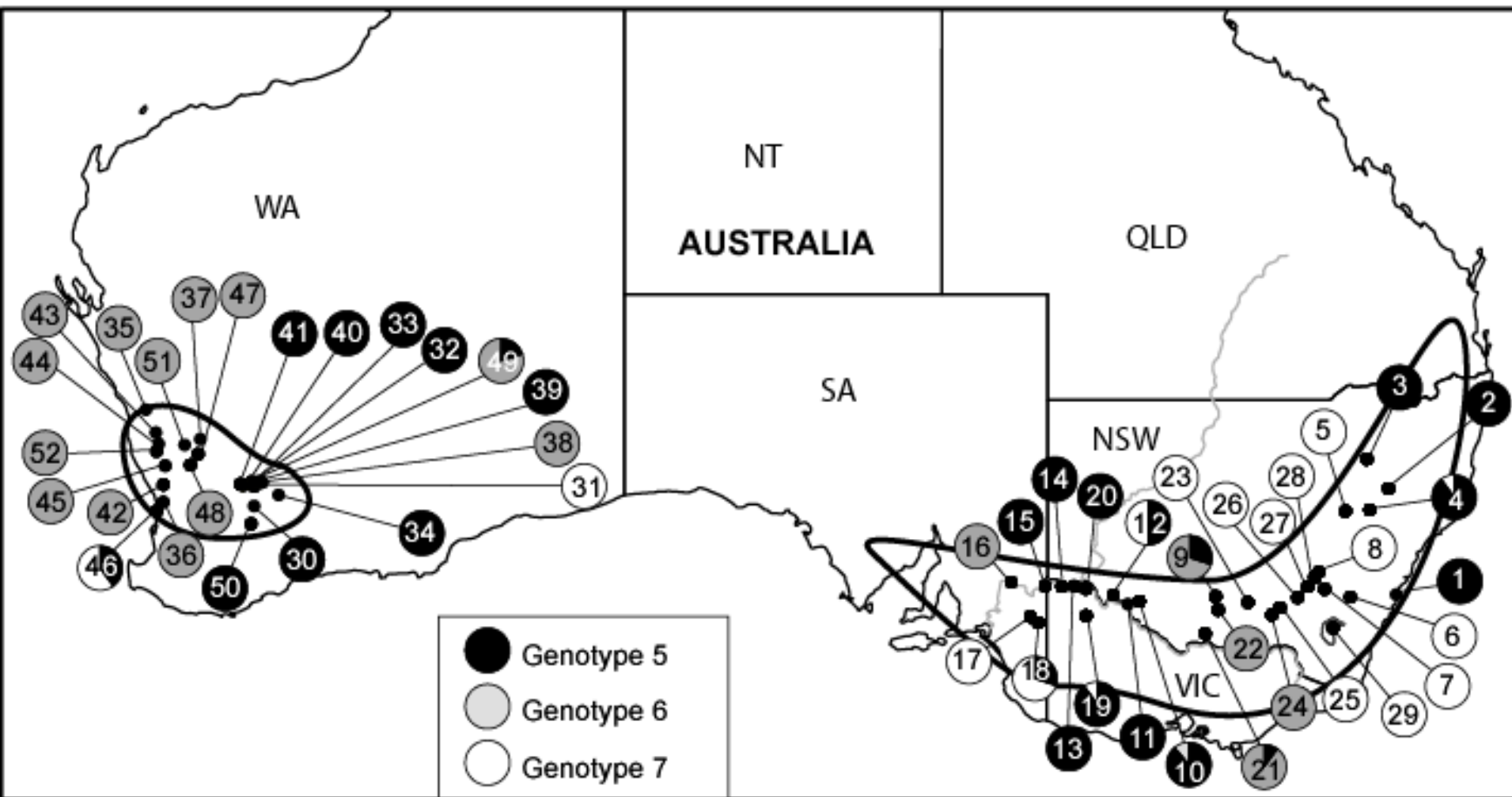


Phenotype variation not as useful, hard to compare across continents.

What we did.

- 1156 plants from invaded countries
 - USA
 - Canada
 - Australia
 - Argentina
- 71 variable AFLP loci (DNA fingerprint)

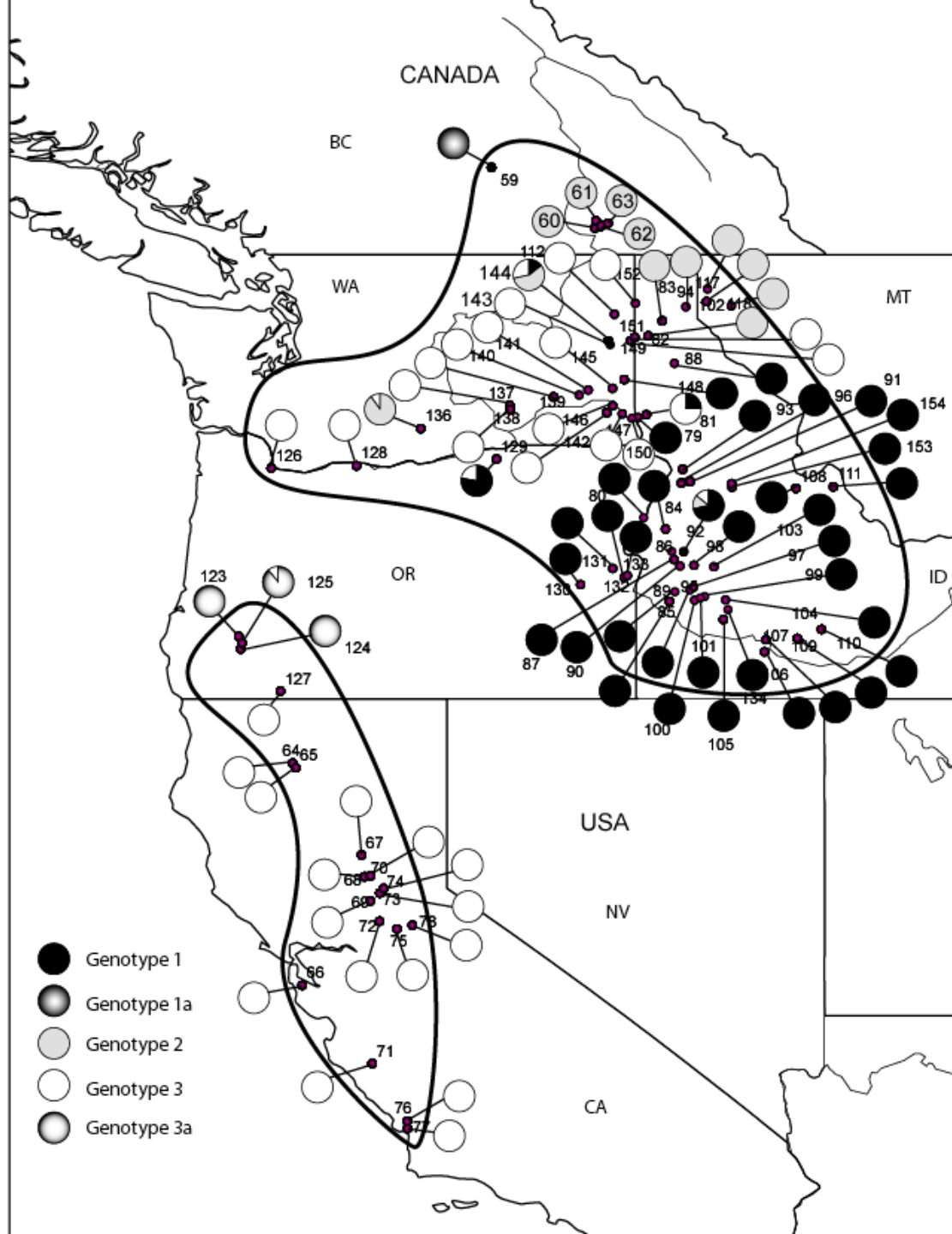
Fig. 1b



377 plants; 52 populations; 3 genotypes

Improvements for Australia

- Demonstrated that 3 genotypes still exist in good numbers (6:4:5 ratio)
- Demonstrated distribution of genotypes



721 plants
96 populations
5 genotypes in
West NA

Population structure!

Genotype 2 resistant

Improvements for N America

- Demonstrated strong population structure
- Identified additional genotypes in E USA

Improvements for all invaded continents

- Found a better, more objective way to describe these plants, with high resolution
- Can compare across invasions and look for origins
- Created a greenhouse garden collection of all known genetic diversity in wNA for further research on:
 - host specificity of future agents (current agents not sufficient for control in most cases)
 - Rush skeletonweed ecology
 - Genotyped plants (at U Idaho)



Native Range: Eurasia



Europe

range (Muesel & Jäger 1992):

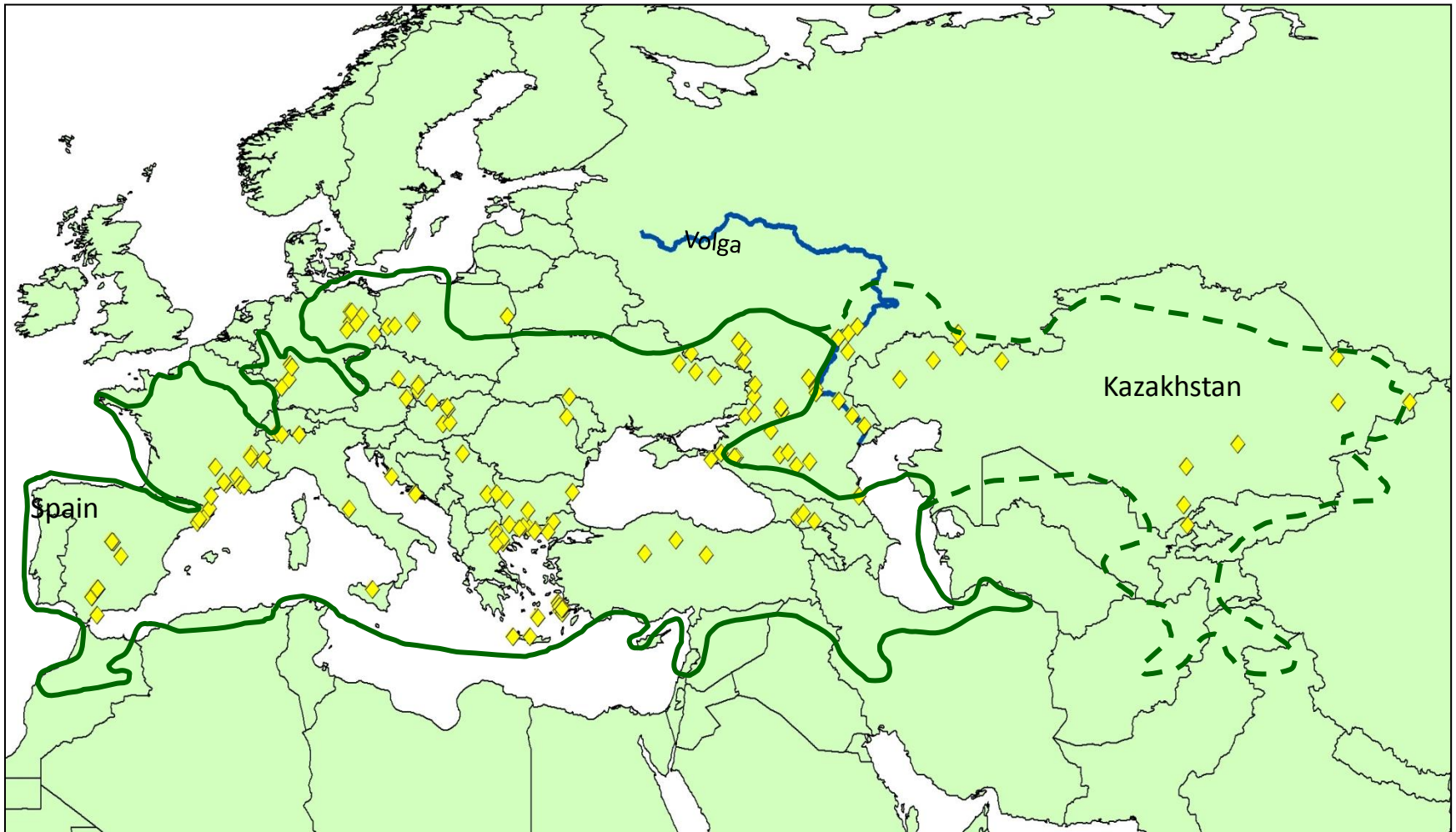
C. juncea sensu stricto



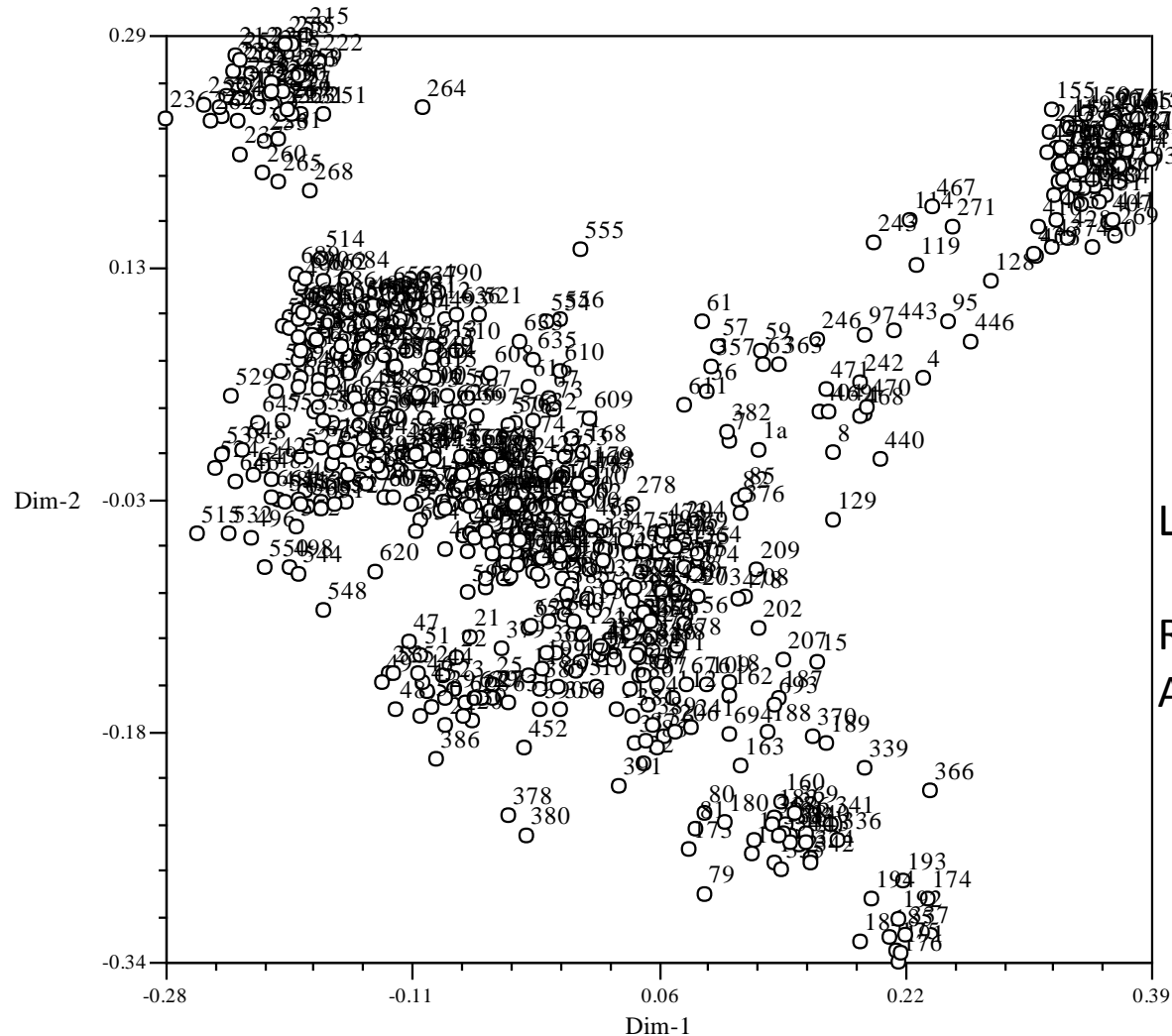
C. juncea sensu lato



collection sites



Europe: 643 plants from 126 pops

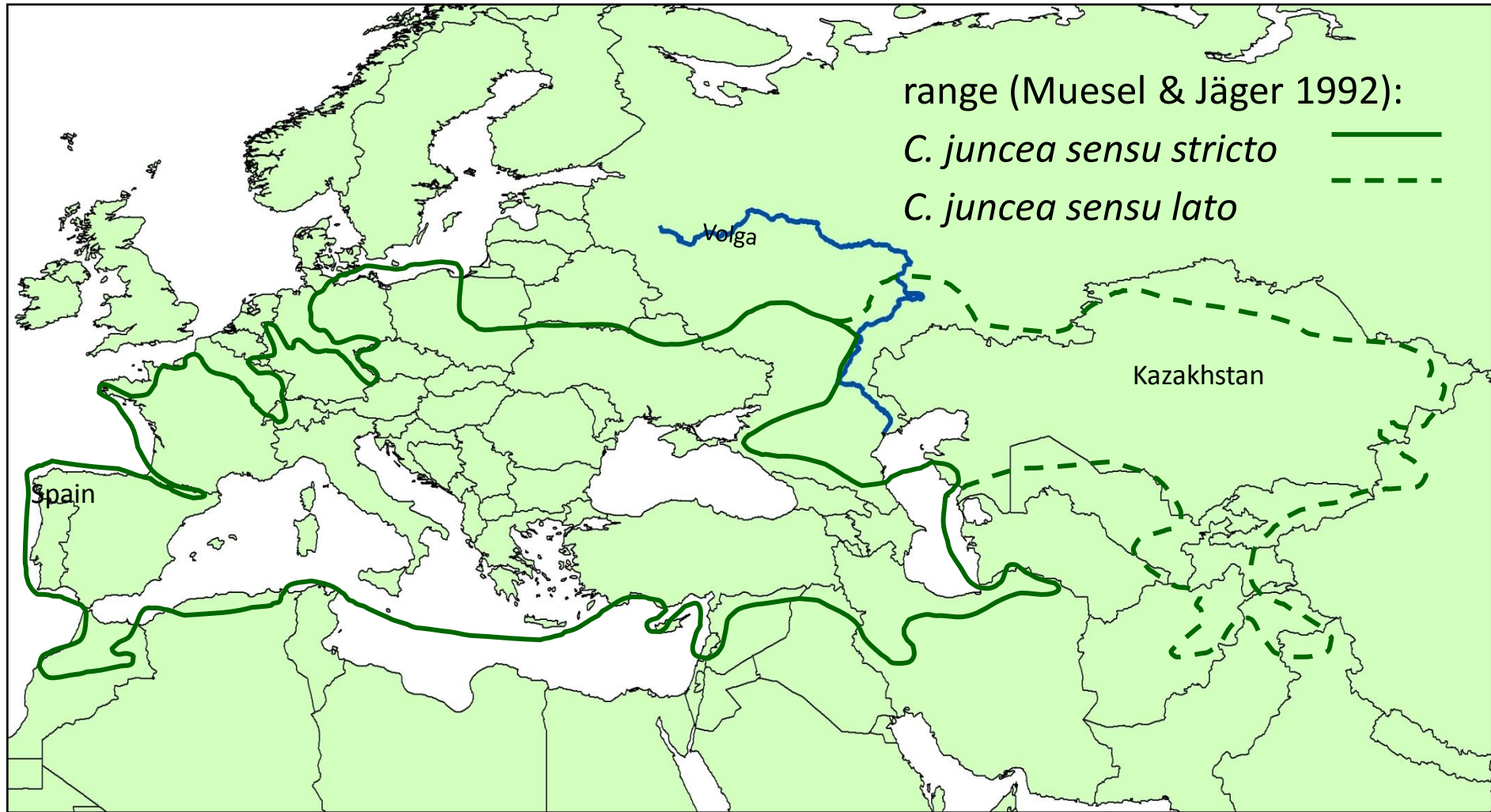


Lots of AFLP Genotypes!

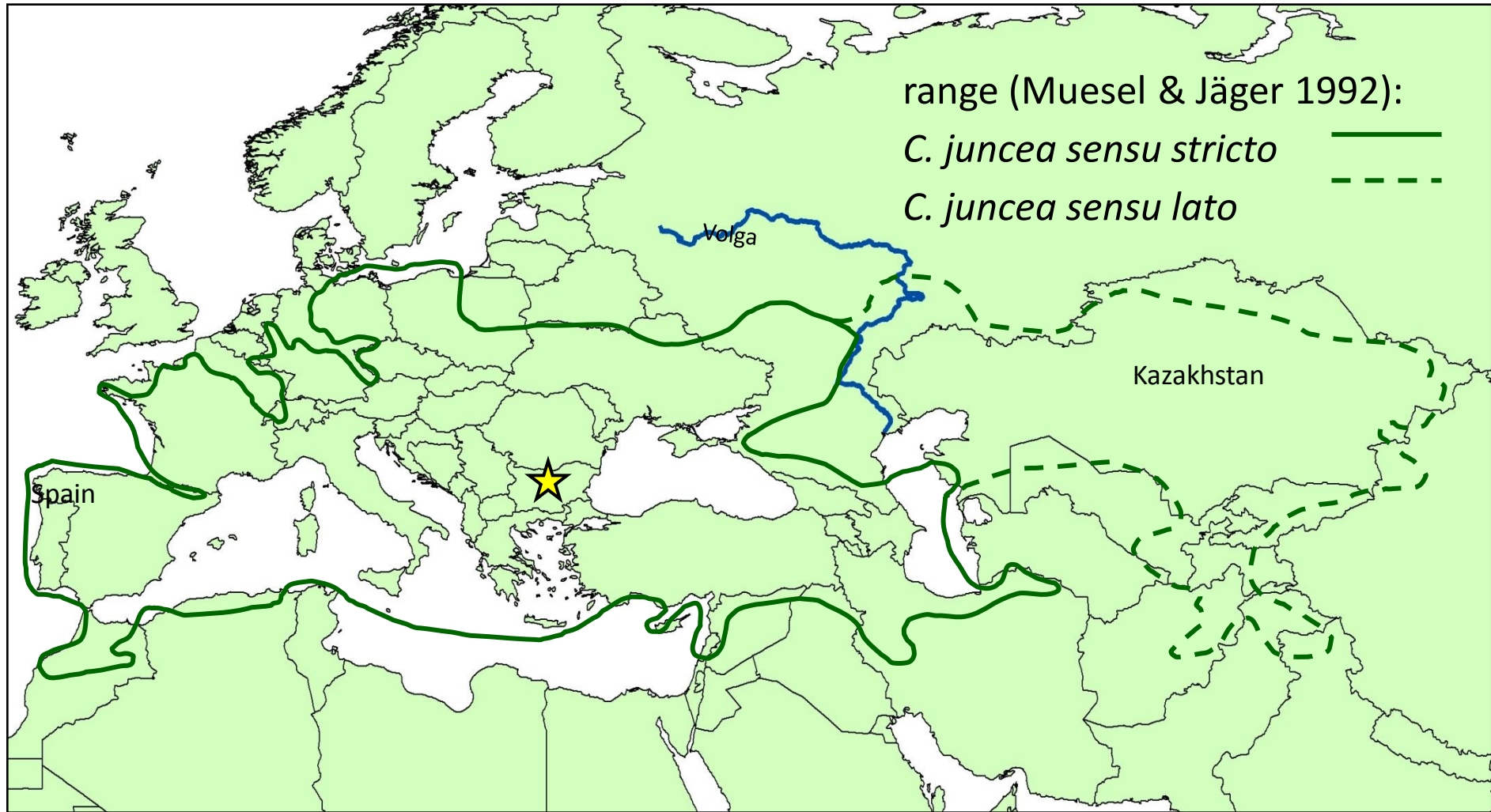
Remember, only 3 in
Australia and 5 in wNA

Improvement over enzyme studies; higher resolution

Origins (prelim data)

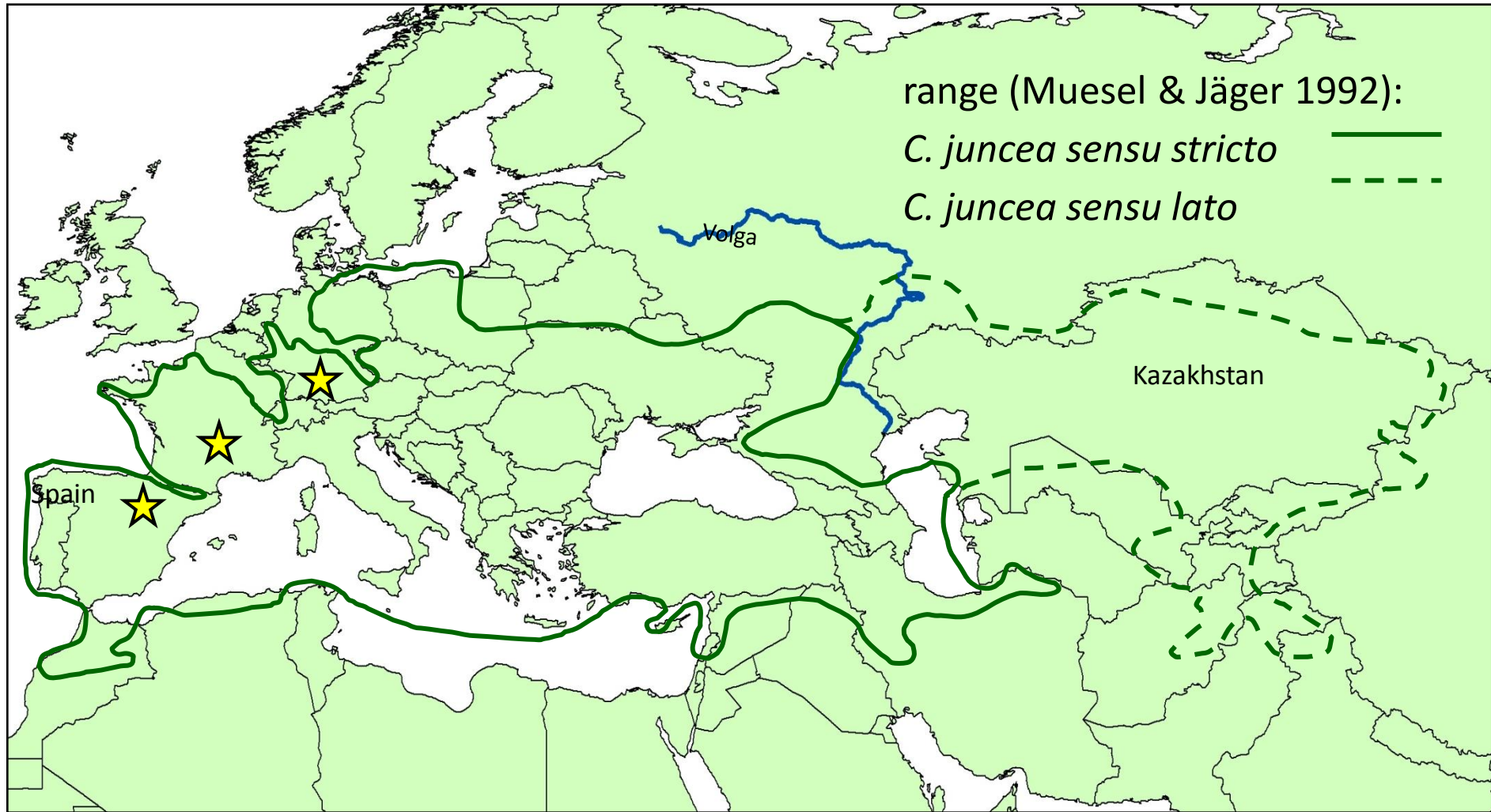


Genotype 1 (USA)

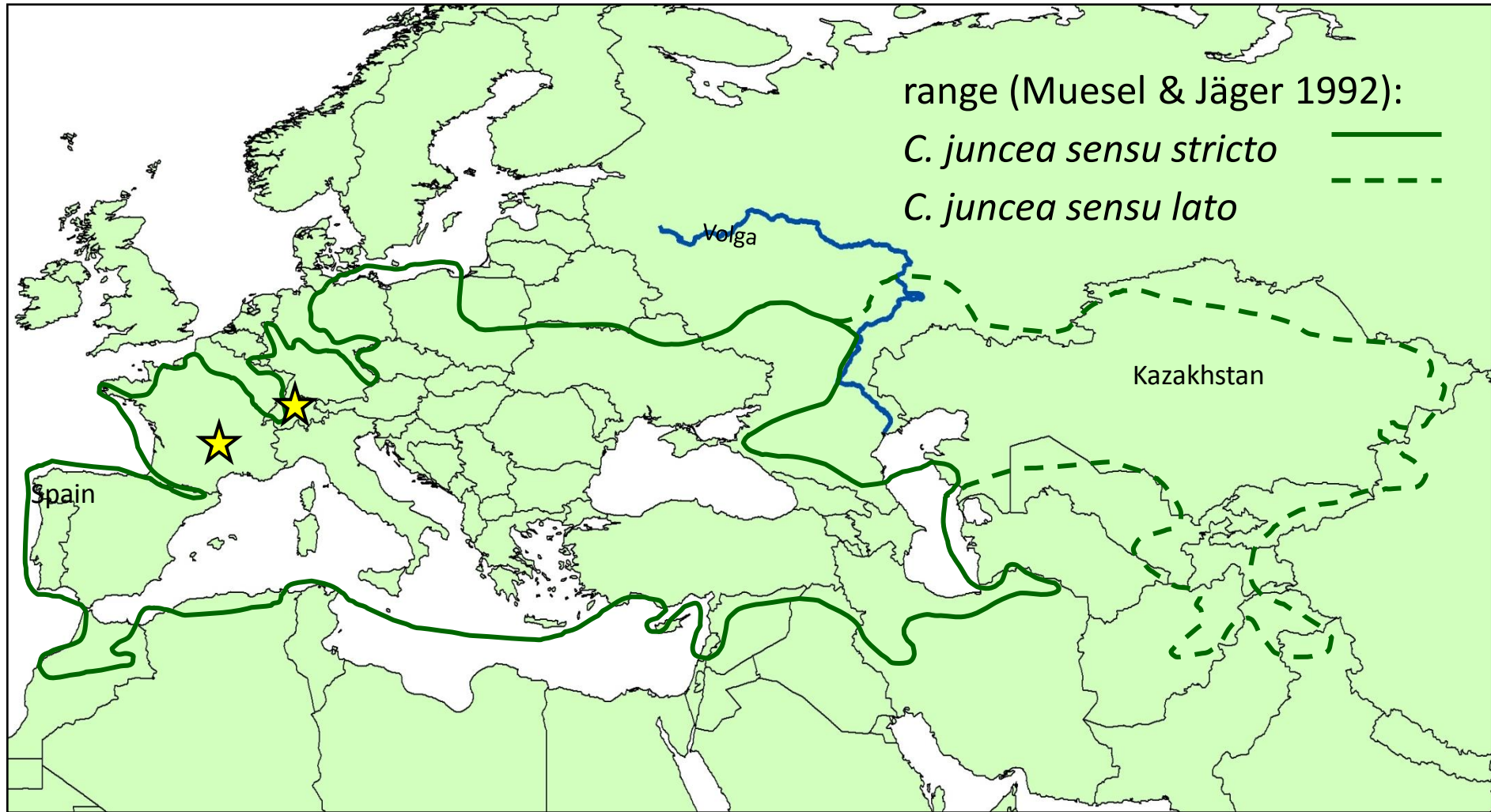


97%, not Turkey or Italy

Genotype 2 (USA)



Genotype 3 (USA)



100% match!

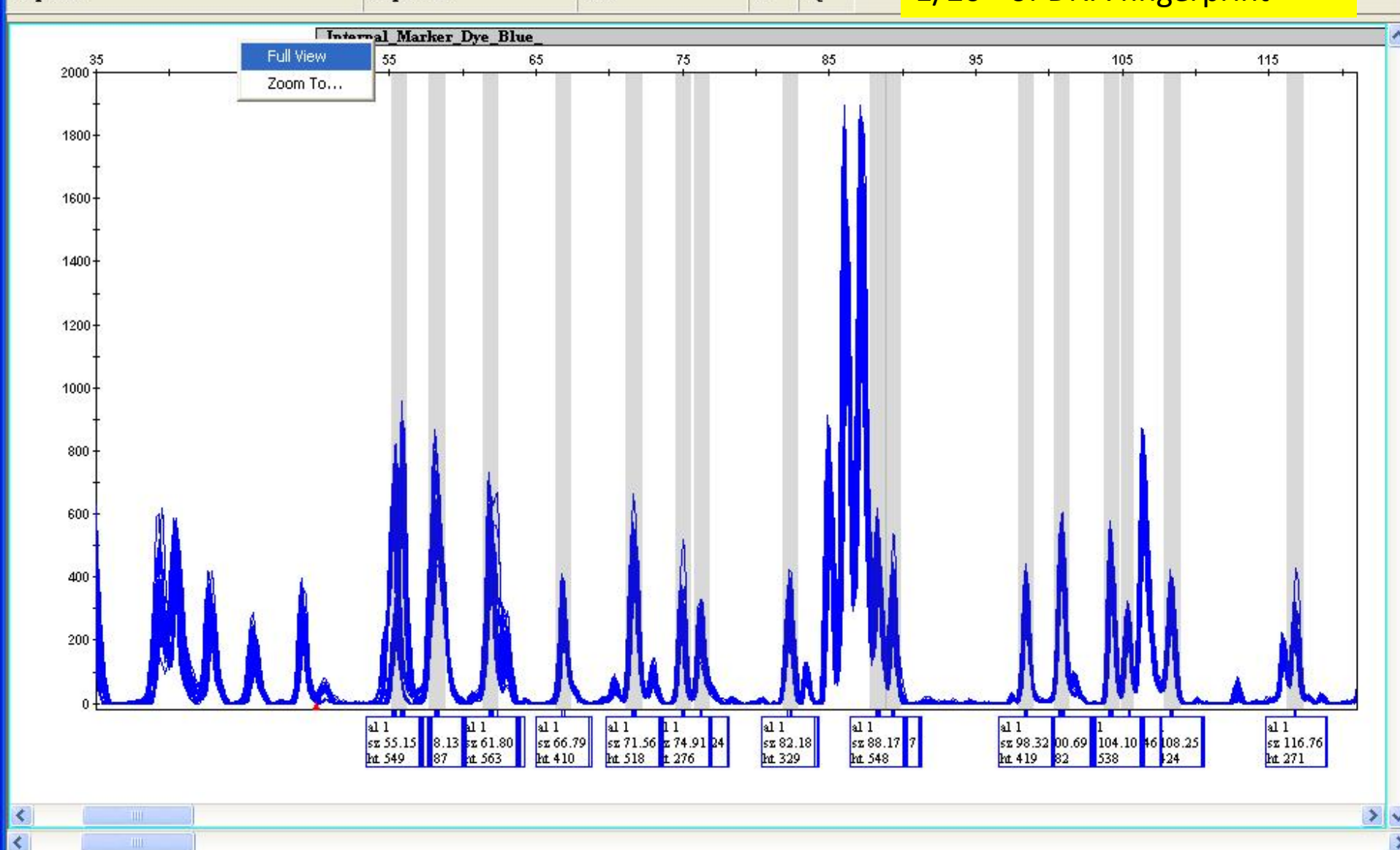
Sample Name

Panel

OS

SQ

1/20th of DNA fingerprint



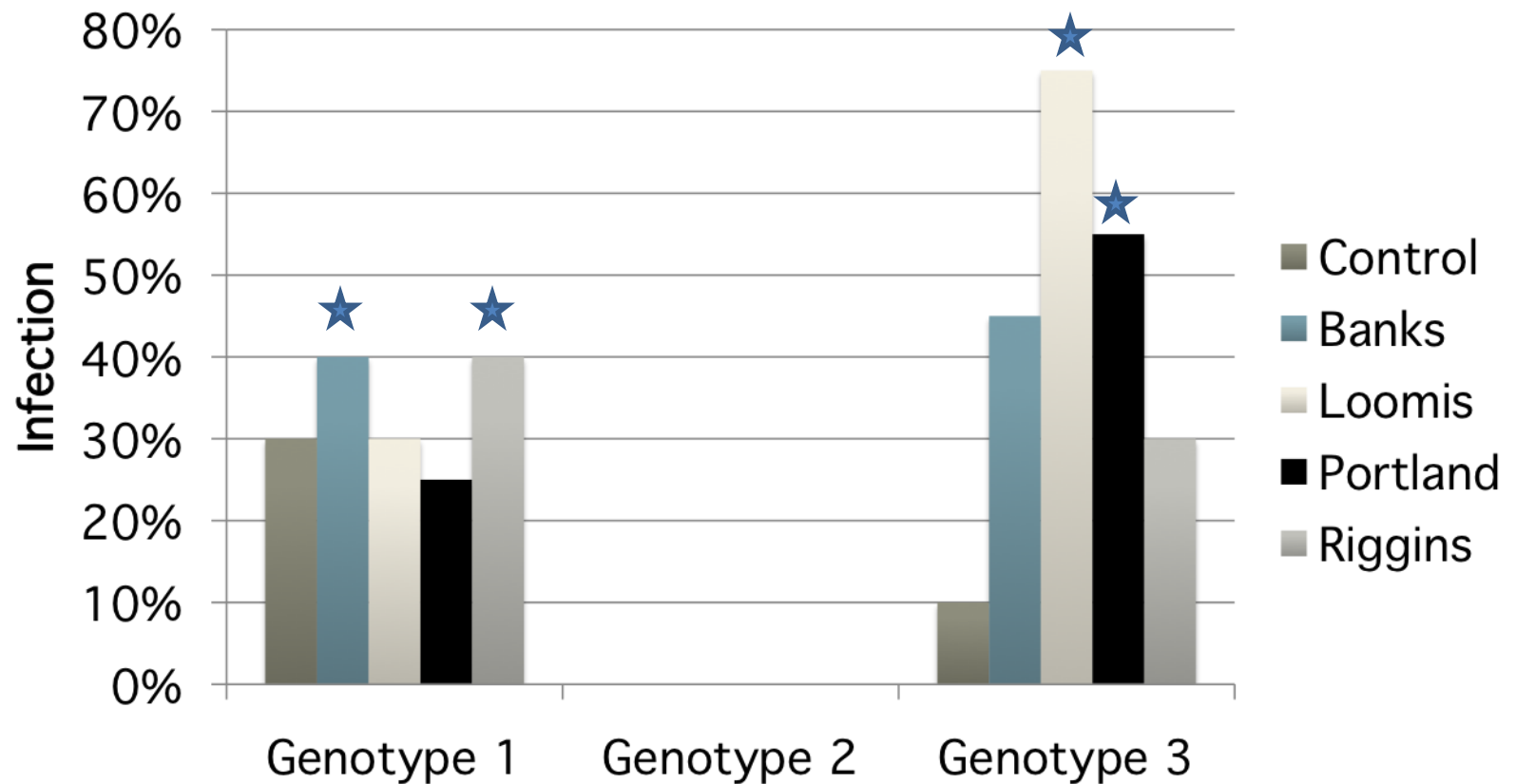
Differences in susceptibility of biotypes of rush skeletonweed to accessions of the skeletonweed rust *Puccinia chondrillina*

Meaghan Bennett, Lisa Collison
Mark Schwarzländer,
Brad Harmon and
Aaron Weed



Results - 21 September 2010

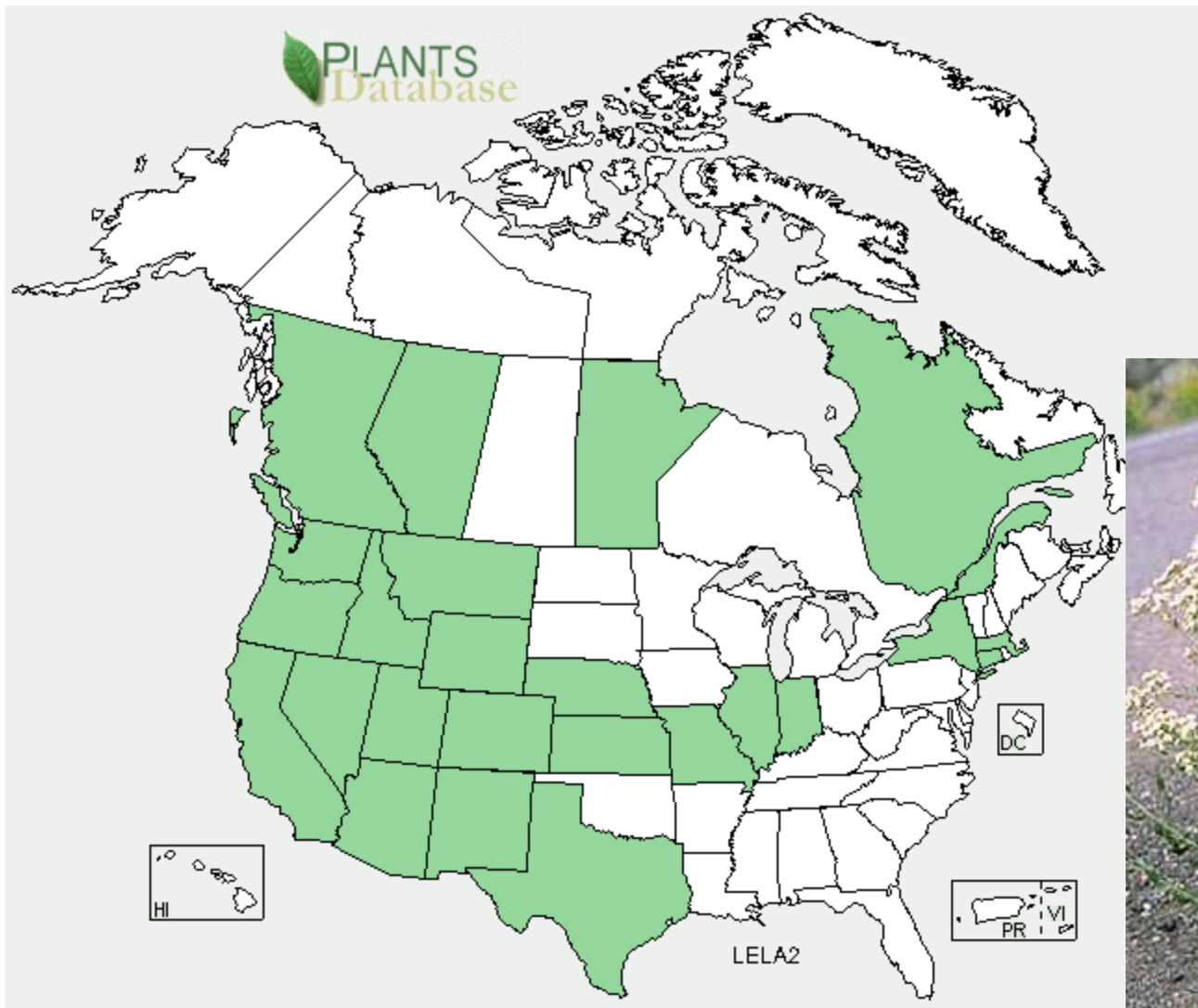
Infection



These field collected fungi are being genotyped.
Original 2 USA rust accessions available

Lepidium latifolium

Perennial Pepperweed



Lepidium latifolium

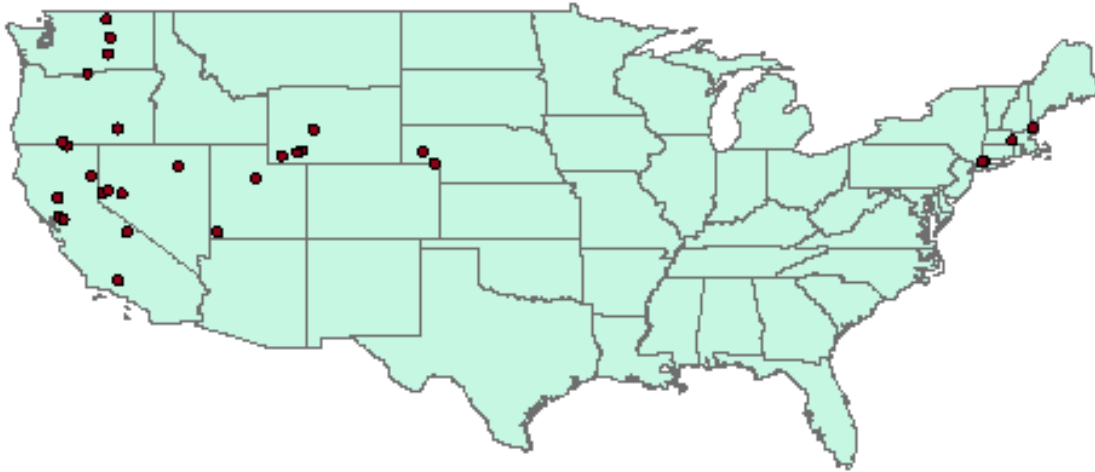
Perennial Pepperweed

- Sexual reproduction (selfing, outcrossing)
- Clonal reproduction (rhizomes)



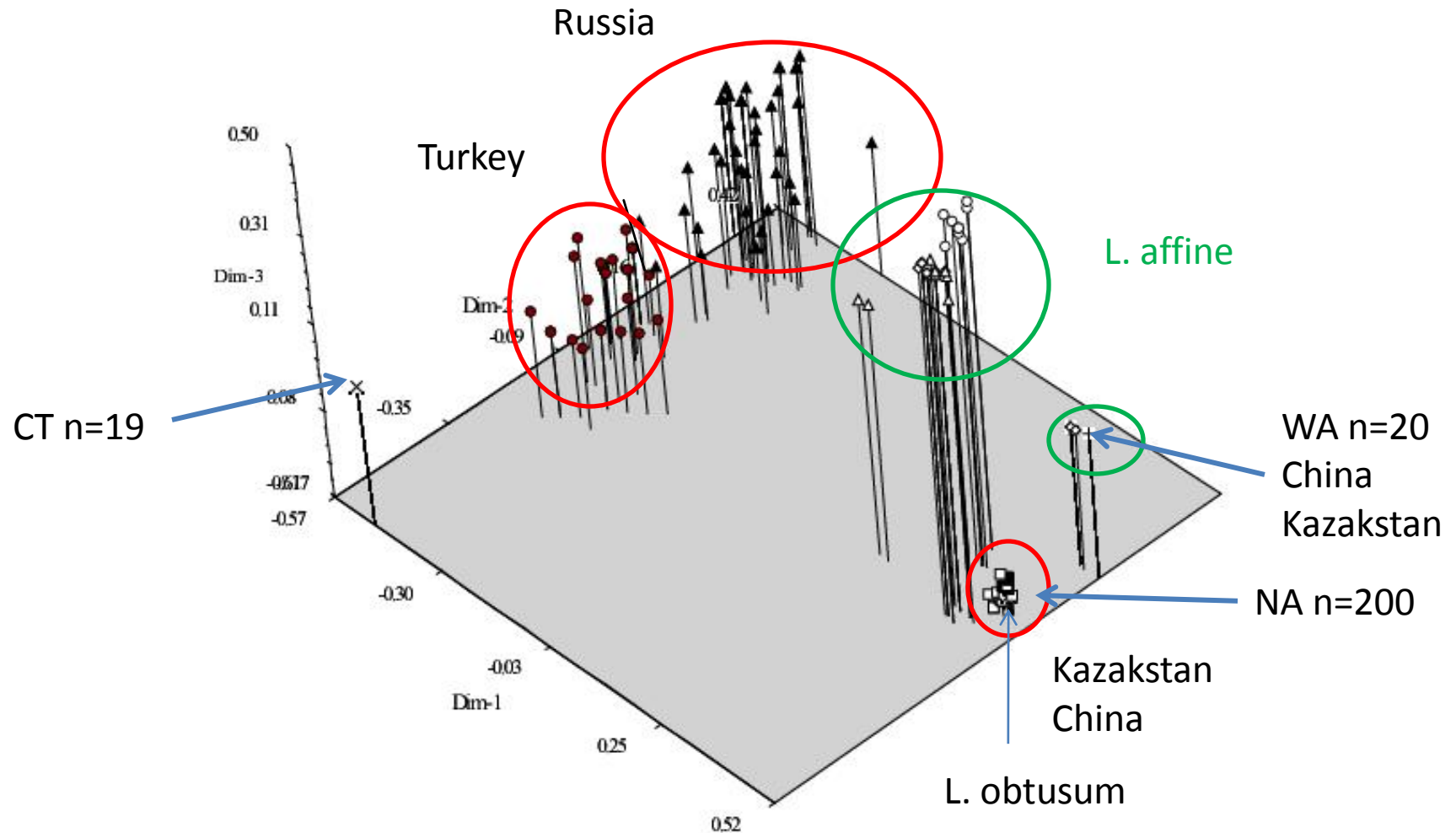
Lepidium latifolium

Perennial Pepperweed

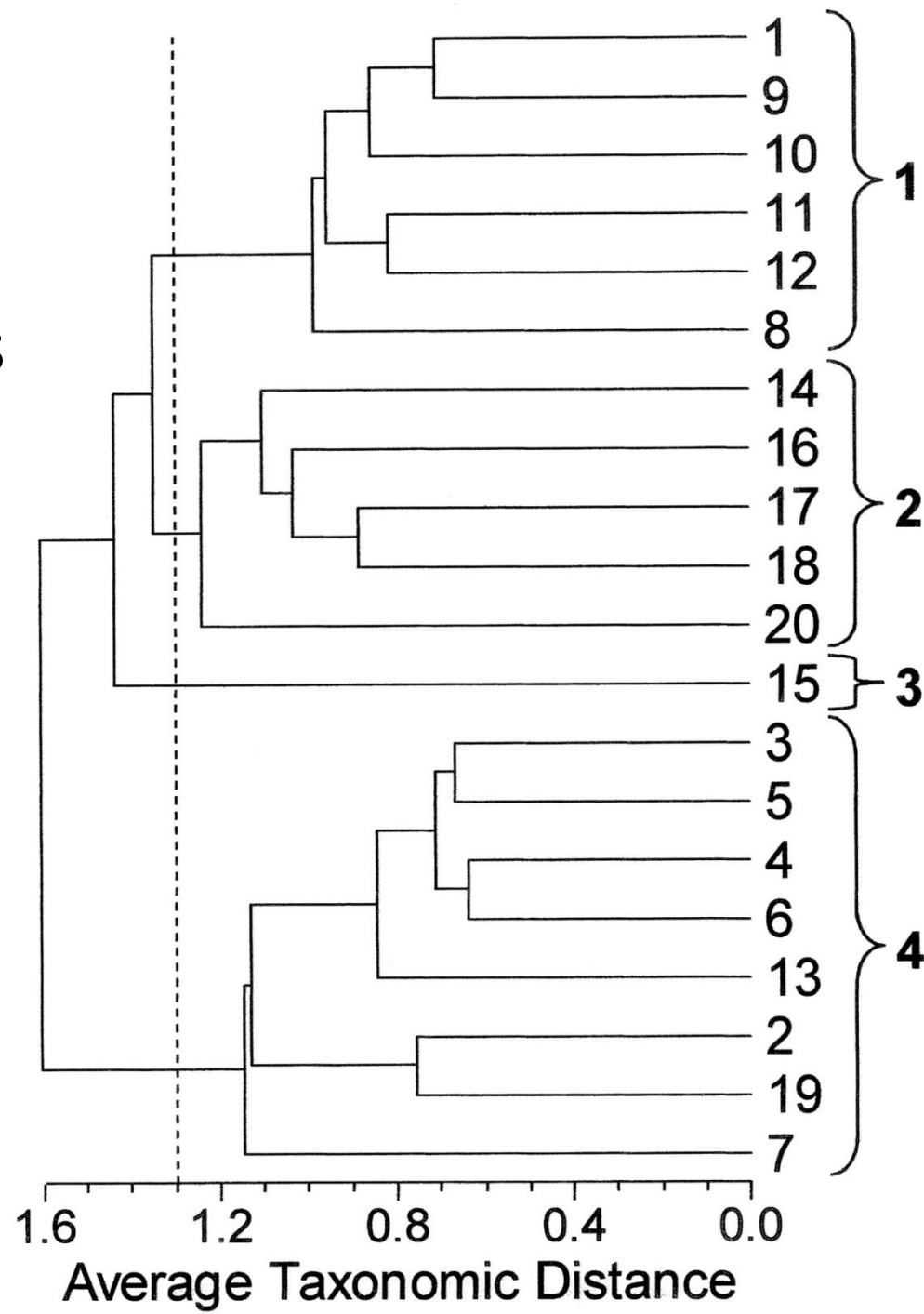


Only 3 AFLP genotypes in USA (n=239 plants)
Invasion involves two species!

L. latifolium



- Numerous genotypes



What does this all mean for biocontrol practitioners and regulation?

- More work
 - Prelim study relatively cheap compared to cost of bc project development or failure
- Better knowledge of origins
 - Increased efficacy
 - Increased establishment
 - Less genotype replacement
 - Release only in susceptible invasion areas
- Better monitoring
 - Can track cryptic phenotypes

Target Taxonomy

- Species origin (tumbelweed; CA)
 - *Salsola kali*, *S. kali* subsp. *austroafricana*, *S. australis*



- Identification of immature targets



Photo by Dr. Wendell Morrill

Host-Specificity Testing of Pathogens with Long Incubation Periods

- Pathogen specific primers can be used to determine infection before symptoms occur.
- Real-time PCR can quantify infection
- *Endophyllum osteospermi* on Boneseed (*Chrysanthemoides monilifera*) in Australia
- Two year period between infection and symptom expression
- Monitoring

Morin, L, Hartley, D., 2008. Proceedings of the 12th ISBCOW



csiro.au



plantzafrica.com

- When does phenotypic or genotypic variation matter?
- Improvements that can be made in understanding and coping with genetic diversity

- A revisit to *Puccinia* and rush skeletonweed



- Applying molecular-based approaches to pathogen-based classical biological control

Eric Coombs, www.forestryimages.org

Questions?